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CANCER-INDUCED MUSCULOSKELETAL DISEASES (E KELLER AND J STERLING, SECTION EDITORS)

Breast Cancer Dormancy in Bone

Miranda E. Clements [1,2] · Rachelle W. Johnson [1,2]

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Abstract

Purpose of Review The goal of this review is to summarize recent experimental and clinical evidence for metastatic latency and the molecular mechanisms that regulate tumor dormancy in the bone.

Recent Findings Tumor dormancy contributes to the progression of metastasis and thus has significant clinical implications for prognosis and treatment. Tumor-intrinsic signaling and specialized bone marrow niches play a pivotal role in determining the dormancy status of bone disseminated tumor cells. Experimental models have provided significant insight into the effects of the bone microenvironment on tumor cells; however, these models remain limited in their ability to study dormancy.

Summary Despite recent advances in the mechanistic understanding of how tumor cells remain dormant in the bone for prolonged periods of time, the signals that trigger spontaneous dormancy escape remain unclear. This review highlights the need for further investigation of mechanisms underlying tumor dormancy using clinically relevant models.

Keywords Tumor dormancy · Breast cancer · Bone metastasis

Introduction

Among women, breast cancer remains the most frequently diagnosed cancer and the second leading cause of cancer deaths [1]. Despite advances in early diagnosis and treatment options, approximately 30% of breast cancer patients will ultimately relapse with metastatic disease caused by the outgrowth of disseminated tumor cells (DTCs) at distant organ sites [2–4]. While metastasis is a highly inefficient process, tumor cells that are able to escape from the primary tumor, survive in circulation, and colonize a secondary site have the potential to form an overt metastasis. Following colonization, DTCs may immediately proliferate into a clinically detectable metastasis or enter a dormant state in which they may remain for an extended period prior to reactivation by intrinsic or microenvironmental cues [5].

Breast cancer exhibits a particularly extensive metastatic latency, defined as the time period between primary tumor detection and metastasis, which suggests a prolonged dormancy stage where DTCs and micrometastases are clinically undetectable [6••]. Intriguingly, estrogen receptor (ER) status correlates with metastatic latency in breast cancer as ER− patients frequently recur rapidly within the first 5 years following diagnosis whereas ER+ patients tend to recur 8–10 years post-diagnosis [7, 8]. Bone metastases occur in ~ 60% of breast cancer patients with metastatic disease, making bone the most frequent site of recurrence followed by the lungs, liver, and brain [9]. Approximately 20% of patients with early-stage disease and no lymph node involvement will eventually develop distant metastases [10]. Further, nearly 70% of breast cancer patients who succumb to disease harbor DTCs in their bone marrow upon autopsy [11, 12]. These findings indicate that a large proportion of patients are at significant risk of developing bone metastatic disease even after long periods without recurrence.

Over the past decade, many studies have focused on expanding our understanding of the temporal and mechanistic regulation of metastatic progression in the bone. However, given the prolonged latency and heterogeneity in tumor cell

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✉ Rachelle W. Johnson
rachelle.johnson@vumc.org

[1] Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[2] Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

behavior, it remains difficult to predict when and which patients will develop metastases. Consequently, therapeutic options for metastatic disease are extremely limited, with the majority being palliative rather than curative. Continued exploration into the underlying biology of tumor dormancy is critical to advance the development of more effective therapies that prevent and/or treat metastasis to ultimately improve patient survival.

Models and Clinical Evidence of Tumor Dormancy

Based on clinical and experimental evidence, two central models of tumor dormancy have been proposed: cellular dormancy and tumor mass dormancy. Cellular dormancy occurs when single DTCs enter a non-proliferative, quiescent state in response to intrinsic signals or a repressive microenvironment. In contrast, tumor mass dormancy represents a state of equilibrium between proliferative and apoptotic DTCs, which is controlled by microenvironmental factors including interactions with stromal cells, vascularization (angiogenic dormancy), or immunosurveillance (immunologic dormancy). Prior to the angiogenic switch that allows tumors to grow beyond ~ 2 mm [13, 14], angiogenic dormancy may occur as a result of enhanced secretion of angiogenesis suppressors and downregulation of pro-angiogenesis factors [15, 16]. During immunologic dormancy, a balance exists between immune clearance of some DTC subpopulations and immune escape of others [17–19]. Over time, DTCs may also adapt to immune system pressures by inducing a protective immune response or altering antigen expression in order to enhance immune escape and tumor growth. Recent experimental evidence suggests that surgery triggers a systemic inflammatory response that results in the outgrowth of DTCs that were once restricted by immunologic dormancy [20]. Importantly, both cellular and tumor mass dormancy likely occur simultaneously within a single patient and potentially even within the same metastatic site, resulting in limited clinical detection of DTCs for extended periods of time.

The detection of circulating tumor cells (CTCs) and DTCs in breast cancer patients has been extensively investigated as a potential predictive indicator of recurrence (recently reviewed in [21]). Increasing evidence suggests that metastatic spread occurs early in tumor progression and likely precedes primary tumor detection [22, 23]. Evidence of early dissemination was demonstrated by the detection of DTCs in the bone marrow of MMTV-PyMT and MMTV-HER2 transgenic mice as well as in patients with ductal carcinoma in situ (DCIS) during pre-invasive stages of tumor progression [22]. These findings were corroborated by other clinical evidence showing DTCs in the bone marrow of patients with early-stage disease [23–25]. Additionally, a clinical study reported the detection of DTCs in the bone marrow of ~ 15% of breast cancer patients without evidence of recurrence and that the presence of DTCs correlated with a higher risk of relapse over the 0–5-year post-diagnosis follow-up (HR 3.1, 95% CI 1.7–5.65, n = 513) [26]. Interestingly, more than half of these patients did not recur within a 10-year follow-up period. More recent clinical evidence indicated that DTC status alone did not correlate with distant recurrence-free survival (DRFS) in early-stage breast cancer patients (n = 273), but was significantly associated with DRFS as well as breast cancer–specific survival (p = 0.0205) and overall survival (p = 0.0168) when combined with CTC status (p = 0.0270) [27]. Together, these findings reveal that DTC detection may provide some insights into metastatic progression but that its utility as a predictor of recurrence is likely cofounded by the complexity of tumor latency.

One of the major challenges to understanding the clinical significance of tumor dormancy is the ability to detect rare DTCs and study their dormancy status in the bone. To date, identification of dormant DTCs in preclinical animal models and patient samples has predominantly relied on the analysis of tumor-specific (cytokeratin, EpCAM), proliferative (Ki67, PCNA), and apoptotic markers (TUNEL, caspase) by flow cytometry and immunocytochemistry. Despite their prevalent use, the currently established methods only provide a glimpse into the dormancy state of DTCs and due to technical limitations cannot efficiently investigate the dynamic nature of metastatic progression. Further development of methodologies to detect and analyze DTCs, particularly in patients, is necessary to better understand the cellular and molecular mechanisms of metastatic latency and clinical recurrence patterns. Recent advances in intravital imaging of tumor cells, including breast cancer cells, within the bone marrow offer promising new approaches to provide further kinetic and mechanistic insights into tumor dormancy [28].

Microenvironmental Regulation of Tumor Dormancy

Increasing experimental evidence suggests that local microenvironmental influences on the primary tumor have persistent effects on tumor cell homing, dormancy status, and chemotherapy resistance in the bone metastatic site. The C-X-C motif chemokine ligand 12 (CXCL12):C-X-C motif chemokine receptor 4 (CXCR4) signaling axis is one of the most well-established mechanisms that promotes breast cancer cell homing and colonization from the primary tumor to the bone [29, 30]. This signaling cascade is often initiated by CXCR4 overexpression on breast cancer cells, making them more susceptible to priming by CXCL12-expressing cancer-associated fibroblasts in the primary tumor and colonization of the CXCL12-rich bone marrow [31, 32]. Hypoxia, or low oxygen

tensions, in the primary tumor has been shown to generate a sub-population of "post-hypoxic" DTCs in breast cancer that, following dissemination, highly express a NR2F1-driven pro-dormancy gene program and are capable of evading chemotherapy [33]. Recently, breast cancer cells that survived adjuvant chemotherapy treatment (doxorubicin and methotrexate) in vitro exhibited sustained activation of the interferon regulatory factor 7 (IRF7)/interferon beta (IFN-β)/interferon alpha and beta receptor (IFNAR) signaling axis, which elicited a state of immunological dormancy and chemotherapy resistance [34]. Together, these findings begin to reveal the significant influence of the primary tumor microenvironment and adjuvant therapy on the subsequent dormancy phenotype and survival of DTCs at distant sites.

The unique nature of the bone marrow as the major site of hematopoietic stem cell (HSC) maintenance and home to many cell types implicated in metastasis plays a significant role in the metastasis of breast cancer cells to the bone (recently reviewed in [35]). The bone marrow represents a particularly permissive microenvironment for the homing and dormancy of DTCs largely due to the presence of two specialized stem cell compartments: the perivascular niche and the endosteal "osteoblastic" niche [36]. Within these niches, resident endothelial cells/mesenchymal stem cells (perivascular niche) and osteoblast lineage cells (endosteal niche) are key producers of survival, quiescence, and self-renewal signals that maintain the HSC population [36]. However, increasing evidence suggests that tumor cells colonize these specialized niches in order to co-opt these microenvironmental signals to promote their own dormancy and survival (Fig. 1a, b) [15, 37, 38].

Using in vivo optical imaging technologies, breast cancer cells were shown to preferentially home to the perivascular niche through interactions with E-selectin-positive endothelial cells [38]. In support of these experimental findings, analysis of patient bone marrow samples revealed that dormant, Ki-67-negative tumor cells were more likely to be found adjacent to sinusoids within the perivascular niche compared to the endosteal surface [38]. Following colonization, resting endothelial cells residing in the perivascular niche secrete thrombospondin-1 (TSP1), an angiogenesis suppressor molecule, which promotes the quiescence of disseminated breast cancer cells [15]. In contrast, pro-angiogenic factors secreted during neovasculature formation such as transforming growth factor beta 1 (TGFβ1) and periostin (POSTN) promote the proliferation of nearby DTCs [15]. Breast cancer cells have also been shown to stimulate mesenchymal stem cell secretion of exosomes containing distinct miRNAs such as miR-222/223 to induce tumor dormancy and therapy resistance [39].

Direct competition of breast cancer cells for either HSC niche has not yet been reported; however, murine models of prostate cancer have shown hijacking of the endosteal niche by tumor cells [37]. These studies demonstrated that experimentally increasing the number of osteoblast lineage cells, thus expanding the endosteal niche, increased the number of DTCs within these niches of the bone marrow. Further, interaction of prostate cancer cells with osteoblasts led to HSC maturation and mobilization out of the niche, allowing for expansion of the DTC population [37]. Direct interaction of N-cadherin-positive osteogenic cells with E-cadherin-positive breast cancer cells has been shown to promote DTC colonization of the endosteal niche [40]. Interestingly, these heterotopic adherens junctions also conferred a proliferative signal through enhanced mTOR signaling, driving the development of micrometastases in the bone [40]. Recent work demonstrated that interaction of breast cancer cells with osteoblasts or bone marrow cells leads to enhanced IL-1B secretion by all cell types, resulting in the expansion of the endosteal niche and tumor cell proliferation [41•]. Thus, the endosteal niche appears to, in part, regulate the dormancy status of DTCs, but it remains unclear how the signals from the endosteal surface impact individual tumor cells and whether this is dependent upon the precise cellular composition of the occupied endosteal niche. Combined, these studies suggest that breast cancer cells often preferentially home to the bone marrow in order to co-opt HSC niches and that promotion or inhibition of DTC dormancy is highly dependent on the microenvironmental context (Fig. 1a, b).

Molecular Mechanisms of Tumor Dormancy

In addition to the consequences of direct microenvironment interactions, numerous tumor-intrinsic signaling pathways have been linked to tumor dormancy (Fig. 1c). One of the most well-described mechanisms promoting tumor dormancy in various cancer types is activation of the p38 MAPK stress-response pathway resulting in a high p38 MAPK to ERK1/2 signaling ratio [6, 42–45]. Microenvironmental factors including bone morphogenetic proteins (BMPs) [46] and TGFβ2 [43, 44] induce $p38^{high}/ERK^{low}$ signaling, resulting in the maintenance of tumor cells in a dormant state in the bone. Interestingly, TGFβ2 was found to be more abundantly expressed in the bone marrow compared to non-skeletal sites such as the lung and liver, suggesting it may represent a particularly potent mechanism of tumor dormancy induction in the bone [47]. Using an in vivo genome-wide shRNA screen, a downstream mediator of p38 MAPK signaling known as mitogen- and stress-activated kinase 1 (MSK1) was recently identified as an important regulator of metastatic dormancy and progression in bone-disseminated breast cancer [48••]. MSK1 downregulation altered chromatin structure leading to reduced expression of luminal differentiation genes (GATA3, FOXA1) and promoted colonization of tumor cells in the bone. Of clinical importance, MSK1 expression or a p38 MAPK-regulated gene signature was associated with

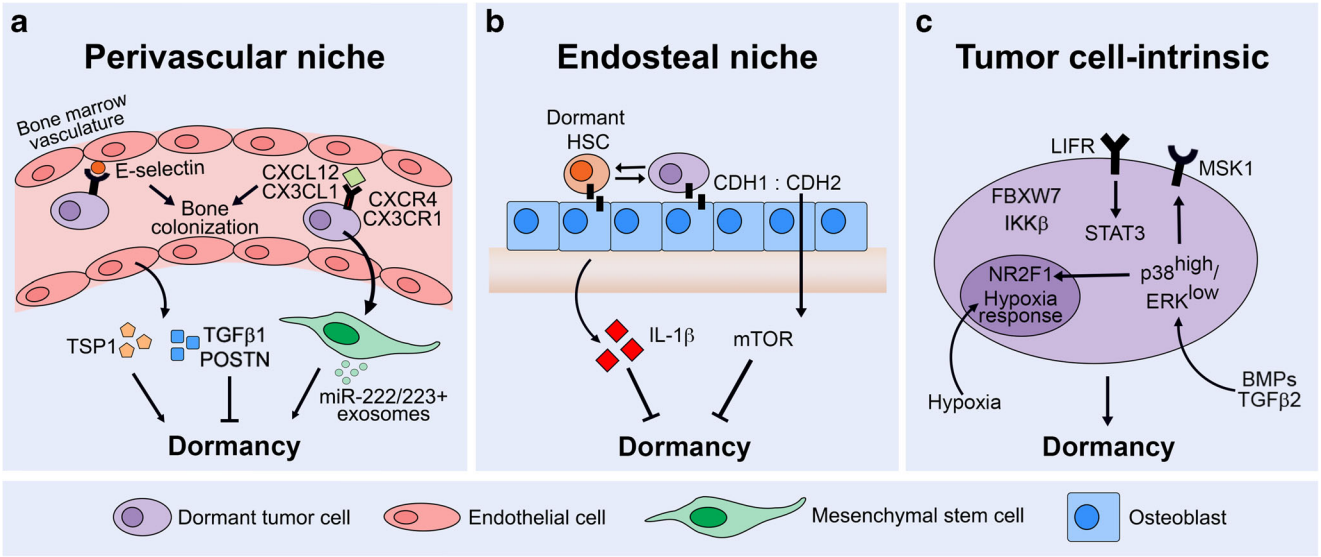

Fig. 1 Mechanisms regulating tumor dormancy in the bone. (**a**) Disseminated tumor cells (DTCs) home to the perivascular niche via bone microenvironmental factors such as CXCL12:CXCR4 and E-selectin. Within the perivascular niche, angiogenesis suppressors such as thrombospondin-1 (TSP1) promote tumor dormancy whereas pro-angiogenic factors including transforming growth factor beta 1 (TGFβ1) and periostin secreted from neovasculature induce tumor cell proliferation. DTCs prime mesenchymal stem cells to secrete exosomes containing miR-222/223 to maintain tumor cells in a dormant state. (**b**) DTCs residing in the endosteal niche co-opt the hematopoietic stem cell (HSC) niche to promote their survival and dormancy. Secretion of IL-1β and activation of mTOR signaling by CDH1:CDH2 interactions between osteoblasts and tumor cells stimulate tumor cell proliferation. (**c**) Tumor cell–intrinsic signaling pathways such as leukemia inhibitory factor receptor (LIFR), p38, and a hypoxia gene signature maintain tumor cells in a dormant state. CXCL12 = CXC chemokine ligand 12; CXCR4 = CXC motif chemokine receptor 4; CX3CL = CX3C motif chemokine ligand; CX3CR = CX3C motif chemokine receptor; TSP1 = thrombospondin-1; TGFβ1 = transforming growth factor beta 1; POSTN = periostin; HSC = hematopoietic stem cell; CDH1 = E-cadherin; CDH2 = N-cadherin; IL-1β = interleukin 1β; mTOR = mammalian target of rapamycin; LIFR = leukemia inhibitory factor receptor; STAT3 = signal transducer and activator of transcription 3; MSK1 = ribosomal protein S6 kinase A5; FBXW7 = F-box and WD repeat domain containing 7; IKKβ = inhibitor of nuclear factor kappa B kinase subunit beta; NR2F1 = nuclear receptor subfamily 2 group F member 1; p38 = mitogen activated protein kinase 14; ERK = extracellular-signal regulated kinase; BMP = bone morphogenetic protein; TGFβ2 = transforming growth factor beta 2

late metastasis in breast cancer patients [42, 48••, 49]. The orphan nuclear receptor NR2F1 is another mediator of p38 MAPK activation that has been shown to promote a pro-dormancy gene signature in numerous cancer types including breast [50]. Recent clinical evidence revealed that breast cancer patients who recurred within 1 year of initial diagnosis harbored < 1% DTCs expressing high NR2F1 while those who did not recur had > 50% $NR2F1^{high}$ DTCs [51]. Despite being a small cohort (~ 20 patients), low NR2F1 expression was significantly associated with reduced time to relapse/breast cancer death ($p = 0.023–0.007$) [51]. Interestingly, no survival difference was observed when DTCs were stratified by the classically used Ki-67 expression. Autophagy, another stress-response pathway, has emerged as a critical mechanism for the maintenance of dormant breast cancer cells in the lungs [52•] as well as in models of ovarian cancer and gastrointestinal stromal tumors [53]. Using 3D in vitro and in vivo lung metastasis models, inhibition of autophagy resulted in significantly reduced cell survival and metastatic burden [52•]. The contribution of autophagy to dormancy in the bone has not been investigated; however, these findings further establish the importance of stress-response pathways to tumor dormancy status.

Considering the bone is a particularly hypoxic environment with oxygen tensions ranging from < 1% to 6% compared to 2–9% in most normal tissues [54], the role of hypoxia signaling in tumor dormancy has recently become of interest. Using the BALB-neuT mouse mammary carcinoma model, genetic analysis of tumors formed by re-inoculation of bone marrow–derived DTCs revealed significant enrichment of hypoxia-related genes compared to parental mammary tumors [55]. Isolated DTCs retained this hypoxia gene signature and HIF-1α was required for DTC tumorigenic potential and outgrowth of tumors [55]. Notably, these experiments were performed using subcutaneous implantation of tumor cells, which partially limits the relevance to bone since the tumor cells were not exposed to the physiological bone microenvironment. The leukemia inhibitory factor (LIF) receptor (LIFR) was recently identified as an activator of tumor dormancy that becomes downregulated under hypoxic conditions resulting in dormancy escape [56, 57]. Experimental mouse models of bone metastasis revealed that loss of LIFR and downstream STAT3 signaling pushed DTCs out of dormancy resulting in enhanced osteolytic bone destruction [56]. Immunohistochemistry of bone metastases revealed that proliferative tumor cells co-localized with the hypoxia probe

pimonidazole whereas seemingly dormant tumor cells stained weakly for pimonidazole [56]. Together with in vitro hypoxia studies, these findings suggest that LIFR signaling confers a dormancy phenotype but may eventually become switched off by hypoxic conditions leading to metastatic outgrowth. Hypoxia has also been implicated in the regulation of TSP1, which has been demonstrated to promote dormancy [15], albeit with conflicting results. Hypoxic conditions were shown to suppress TSP1 expression in various normal and transformed cells [58, 59] while hypoxia-driven TSP1 stimulation was observed in models of prostate cancer [60] and pulmonary hypertension [61, 62]. Thus, further investigation into the hypoxic regulation of TSP1 and the contribution of other factors including tissue-specific microenvironments or oncogenic signaling, particularly in the bone, is necessary. Intriguingly, hypoxia appears to play a dual role in tumor dormancy by inducing a pro-dormancy effect in the primary tumor as discussed earlier while most evidence suggests it promotes DTC dormancy escape in the bone marrow.

The E3 ubiquitin ligase component FBXW7 was recently identified as a pro-dormancy factor given that its deletion in tumor cells resulted in more proliferative bone DTCs in vivo compared to the control group [63]. Further, loss of FBXW7 sensitized tumor cells to chemotherapy suggesting a potential therapeutic strategy to reactivate and eradicate DTCs [63]. Of important note, these studies were performed using genetic ablation as there are no pharmacological inhibitors; however, development of such inhibitors will be essential for this mechanism of eradication to be clinically meaningful. Similarly, activation of inhibitor of nuclear factor kappa B kinase subunit beta (IKKβ) signaling was shown to overcome E2-stimulated proliferation by inducing a quiescent phenotype while also cooperating with E2 to promote tumor cell survival [64]. Our laboratory has previously shown that overexpression of parathyroid hormone–related protein (PTHrP) in breast cancer cells results in downregulation of a number of dormancy-associated genes [56, 65] and rapid osteolytic bone destruction in vivo [66]. The effect of PTHrP overexpression on dormancy occurs through a non-canonical PTHR1/cAMP-independent mechanism that may be associated with the calcium signaling pathway [65]. Interestingly, it was recently demonstrated that the osteogenic niche serves as a calcium reservoir for bone DTCs and that increased intracellular calcium promotes the progression of bone metastases [67]. However, further investigation of the calcium signaling pathway will be necessary to determine its functional role in PTHrP-driven dormancy escape in the bone.

Stem cell–like characteristics have also been linked to the maintenance of tumor cells in a dormant state. Following intracardiac injection and several months of latency of lung and breast cancer cells, isolated "latency competent cancer (LCC) cells" from the lungs, kidney, and brains were found to highly express a mammary stem cell gene signature including the Sox2 and Sox9 transcription factors [19]. Further investigation revealed that the WNT inhibitor DKK1 promotes immunologic dormancy of tumor cells resulting in immune evasion [19]. Notably, LCC cells were able to form bone metastases upon re-inoculation suggesting that these intrinsic dormancy cues may also be relevant to the bone; however, this has not yet been experimentally tested. Similarly, the previously mentioned p38 MAPK gene signature that correlates with metastatic recurrence includes numerous pro-dormancy genes such as NR2F1, TGFB2, and DNMT1, which are known epigenetic modulators of stem cell quiescence [42, 49].

Models to Investigate Tumor Dormancy

One of the major limitations to studying tumor dormancy is the lack of reliable, clinically relevant models. Numerous in vitro and in vivo models have been established in order to study the effects of the bone marrow microenvironment on breast cancer cell behavior and metastatic progression (Table 1). Transgenic models such as MMTV-PyMT and MMTV-HER2 provide the opportunity to study spontaneous metastasis to bone and have shown that dissemination of tumor cells occurs in parallel with primary tumor development [22]. DTCs enter an indolent state prior to reactivation in the bone marrow in these models [22]. However, transgenic models often require many months to detect DTCs, do not reliably metastasize to the bone, or require surgical removal of the primary tumor to allow enough time for establishment of micrometastatic disease. While these models quite accurately recapitulate the prolonged latency periods observed in the human condition, the majority of in vivo studies rely on the inoculation of tumor cells directly into the circulation through the left cardiac ventricle [48••, 56, 76, 77], iliac artery [78, 79], or tail vein [80, 81]. Importantly, these experimental metastasis models mimic bone colonization rather than the entire metastatic cascade and often utilize highly aggressive cell lines that develop metastases without a latency period [35, 82]. Together, the aforementioned limitations make it difficult to investigate tumor dormancy and metastatic outgrowth in these models.

The human ER+ MCF7 cell line has been proposed by several groups to be a clinically relevant model of tumor dormancy in the bone as it exhibits a prolonged latency period and induces little osteolytic bone destruction in vivo [49, 56, 83]. A similar latency tumor model was recently reported using a dormant bone metastatic line derived from the human ER+ T47D breast cancer cell line that develops overt bone metastases ~ 8 weeks post-inoculation [48••]. The physiological relevance of the MCF7 and T47D models is limited given their requirement of exogenous estrogen (E2) for the development of metastases, which has known adverse effects on the

Table 1 Models to study tumor dormancy

Model system	Description	Examples
In vitro 3D models	Co-culture of breast cancer cells with various bone marrow cells	Co-culture in 3D-collagen biomatrix [68], co-culture in 3D endosteal niche [69], 3D spheroid co-culture [70], 3D microvascular networks [15]
Experimental bone metastasis models	Latent DTCs can be detected in the bone following intracardiac inoculation and tumor colonization. Tumor cells exhibit a prolonged latency period prior to the development of overt metastases	MCF7 [56, 71], T47D DBM [48], SSM2 and SSM3 [72], H2087-LCC and HCC1954-LCC [19], HMT-3522-T4-2 [15]
Patient-derived xenograft (PDX) models	Dormant tumor cells derived from PDX primary tumors have been identified in the bone marrow of mice	Triple-negative PDX models [73]
Transgenic mouse models	Tumor cells spontaneously disseminate and can be detected as latent micrometastases in the bone marrow	MMTV-PyMT [74, 75], MMTV-HER2 [74, 75]

bone and urinary tract system [84, 85]. Using highly sensitive flow cytometric and quantitative PCR approaches, our laboratory recently reported that E2 promotes MCF7 growth in the bone following intracardiac inoculation, but is not necessary for initial colonization of the bone [71•]. These findings provide an opportunity to investigate the tumor dormancy phenotype and microenvironmental influences without confounding E2 effects. Additionally, we established that the ER− human SUM159 and ER+ syngeneic murine D2.0R cell lines may also serve as novel models of bone colonization in which to investigate prolonged latency [71•]. The first ER+ mouse mammary carcinoma models that spontaneously metastasize to the bone consistently were reported using the SSM2 and SSM3 cell lines derived from spontaneous tumors formed in STAT1 knockout mice [72]. These models represent a significant advancement to the field as they use immunocompetent syngeneic mice and, while not directly studied, appear to exhibit a prolonged latency period up to 7 weeks prior to the detection of overt metastases. Dormant DTCs have also been detected in patient-derived xenograft (PDX) models [73]; however, these models have not been extensively used to investigate mechanisms of tumor dormancy. The limited use of PDX models is partly due to the low engraftment rate, lack of an intact immune system, and the presence of murine rather than human stroma [86]. Additionally, bone metastases are rarely observed in PDX models despite the high prevalence in breast cancer patients [87, 88].

Several groups have aimed to develop 3D cultures that recapitulate the in vivo bone microenvironment in order to more efficiently study tumor dormancy. One of the first in vitro models was developed using a 3D-collagen biomatrix to co-culture breast cancer cell lines with various cell types abundant in the bone marrow [68]. Using this model, the authors described an "inhibitory niche" established by immortalized cell lines representing osteoblasts, mesenchymal cells, and endothelial cells versus a "supportive niche" driven by primary bone marrow stromal cells. Depending on the niche, tumor cells were induced into a dormant (inhibitory niche) or proliferative (supportive niche) state, which was reversible either by changing the microenvironmental conditions or inhibiting known dormancy pathways such as p38 MAPK and TGF-β [68]. Similarly, recent work established a 3D bone endosteal niche model containing endothelial cells, bone marrow stromal cells, and fetal osteoblasts in a collagen matrix that maintains breast cancer cells in a dormant state [69]. This model was used to identify *BHLHE41*, *HBP1*, and *WNT3* as novel and potentially targetable factors that control tumor dormancy [69]. Additionally, a 3D spheroid co-culture system with tumor cells and bone marrow stromal cells was recently reported as a method to analyze the growth phase and therapeutic response of breast cancer cells [70]. While this study monitored cell cycle status using fluorescent ubiquitination-based cell cycle indicator (FUCCI) expressing cells, the FUCCI system does not allow for efficient monitoring of quiescent, non-proliferative states.

Taken together, while these models have provided significant insight into the effects of the bone marrow microenvironment on breast cancer cells, they are still limited in their ability to study tumor dormancy. One constraint of these models is the unlimited access of tumor cells to microenvironmental factors such as oxygen and nutrients, which does not mimic in vivo conditions. Integration of microfluidic- and bioreactor-based systems into these 3D models offers the potential to improve their physiological relevance. Additionally, future studies incorporating immune cells into these models would provide the opportunity to investigate the mechanisms that contribute to immunologic dormancy. Further development of these model systems would provide significant advances to our ability to monitor the dynamic nature of tumor dormancy in physiologically relevant conditions.

Conclusions

Metastatic disease remains a significant clinical challenge due to the limited mechanistic understanding of progression and therapeutic options for bone DTCs. The recent evidence supporting early dissemination of tumor cells and metastatic latency complicates the ability to predict when and which patients will develop metastases. Despite recent advances in our understanding of DTC dormancy, the timing and stimulus for tumor cells to exit dormancy remain unclear since it is technically challenging to monitor these processes in vivo. Additionally, the complexity of the bone microenvironment makes it particularly difficult to identify specific stimuli and which cellular sources contribute to reawakening. Further insights into the molecular underpinnings of tumor dormancy will be essential to identify therapeutic strategies to prevent metastatic progression and ultimately improve patient survival.

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Compliance with Ethical Standards

Conflict of Interest M.E.C. declares no conflict of interest.

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- • Of importance
- •• Of major importance

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